AMENDMENTS TO THE SPECIFICATION

In the specification, at page 10, lines 4-19 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

This has led to the utilisation of various reversible chemical modifications of the peptide main chain, to enhance the *cis* amide bond conformation and hence reduce ring strain upon cyclisation, and to improve cyclisation yields. In the synthesis of cyclo-[Phe Phe Phe Phe] (SEQ ID NO:61), each amide N was substituted with a Boc (Cavelier-Frontin *et al*, 1993). In this instance the cyclisation yield increased from less than 1% to 27%. Similarly, the use of the *N*-(2-hydroxy-4-methoxybenzyl) (HMB) group as a reversible *N*-substituent has resulted in similar increases in yields of cyclic peptides (Ehrlich *et al*, 1996; Ehrlich *et al*, 1996), although no systematic study has been undertaken to quantify these effects. From the point of view of constructing peptide libraries it is impracticable to substitute every amide N of the linear precursor.

In the specification, at page 25, lines 8-12 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Figure 1 shows HPLC elution profiles of the crude product of solid phase synthesis of cyclo-D-G-(Cat)-R-G (SEQ ID NO:1) following cyclisation and concomitant cleavage from the resin (Profile A) and HPLC-purified cyclo-D-G-(Cat)-R-G (SEQ ID NO:1) synthesised in solution phase (Profile B).

In the specification, at page 25, lines 13-16 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Figure 2 shows an LC-MS profile of the crude filtrate obtained after HF cleavage and base cyclisation of a cyclic peptide synthesised using a safety catch linker of n=2. Cyclo-[D-G-Amb-R-G] is SEQ ID NO:50 and cyclo-[D-G-Amb-R-G-D-G-Amb-R-G]is SEQ ID NO:52.

In the specification, at page 26, lines 4-10 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Figure 6 shows an HPLC comparison of the crude cyclisation products of peptide **1f** using either HATU or BOP as cyclisation reagent. The two major peaks in the chromatograms have a molecular weight of 825g/mol, corresponding to the target cyclic product cyclo-[(Hnb)Gly-(Hnb)Tyr-Arg-Phe] (SEQ ID NO:2). The first eluting product is the all-*L* isomer, the second product contains *D*-Phe.

In the specification, at page 26, lines 14-17 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Figure 8 shows results of crude HPLC of linear peptides **17** and **18** using backbone linkage. A = H-Tyr-Arg-Phe-Gly-OH **17** (SEQ ID NO:3); B = **[HnB]**Tyr-Arg-Phe-Gly-OH **18** (SEQ ID NO:4); Cleavage was performed using HF : p-cresol, 9 : 1, -5 °C, 1 h.

In the specification, at page 26, lines 18-23 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Figure 9 shows the results of crude HPLC for the cyclisation of linear peptides **16** using backbone linkage. A = **[HnB]**Tyr-Arg-Phe-Gly-OH **18** (SEQ ID NO:4); B = cyclo-[**[HnB]**Tyr-Arg-Phe-Gly] **21** (SEQ ID NO:5). Cyclisation was performed using BOP, DIEA, 3 days, while cleavage was performed using HF : p-cresol, 9 : 1, -5 °C, 1 h.

In the specification, at page 26, lines 24-36 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Figure 10 shows the effect of compounds (1μM) on evoked excitatory junction currents (measure of transmitter release) from sympathetic varicosities of the mouse vas deferens. Each filled circle represents an EJC recorded during 100 minutes. Failure to record an EJC is indicated by filled circles on zero of the y-axis. The lower horizontal line indicates when the mixture of cyclic tetrapeptides (1μM) was applied to the tissue bathing solution and the upper horizontal line when naloxone (1μM) was added to the tissue bathing solution. Note that the mixture of tetrapeptides (1μM) greatly reduces the EJC amplitude and frequency, and that the opiate antagonist (naloxone) inhibits this effect. Cyclo [Tyr-Arg-Phe-Gly] is SEQ ID NO:42.

In the specification, at page 27, lines 1-9 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Figure 11 shows the effect of a mixture of cyclic tetrapeptides (1μM) on the average excitatory junction current (EJC) recorded from sympathetic varicosities of mouse vas deferens. Each bar is the average of at least 60 recordings, and the vertical lines show the standard deviation of the mean. Note there was a highly significant decrease in EJC amplitude and frequency following 20 minutes of cyclic tetrapeptide exposure of the preparation, and that this effect was reversed by naloxone. Cyclo [Tyr-Arg-Phe-Gly] is SEQ ID NO:42.

In the specification, at page 31, lines 1-14 please delete Table 3 and replace with the following Table 3 after implementing the following changes:

Table 3

Linear *N*-substituted Tetraglycines and Corresponding Yields of Cyclisation

Linear tetrapeptide	SEQ ID NO:	Yield of cyclisation
Gly-Gly-Gly-Gly	6	<1%
Gly-Gly-Gly-Sar	7	8%
Gly-Gly-Sar-Gly	8	11%
Gly-Sar-Gly-Gly	9	1%
Gly-Gly-Sar-Sar	10	18%
Gly-Sar-Gly-Sar	11	2%
Gly-Sar-Sar-Gly	12	13% (16%*)
Gly-Sar-Sar-Sar	13	~5%

In the specification, page 31, lines 20-34, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The yield for each cyclisation was calculated from the weight of isolated product. The results of this experiment

suggest that *N*-substitution of the *N*-1 or *N*-2 position of a tetrapeptide significantly improves yields of cyclisation whereas *N*-substitution at the third residue has little effect. The effect of multiple substitution at two or more *N*-sites appears to be more or less additive. The best cyclisation result was obtained with the *N*-1 and *N*-2 substituted precursor Gly-Gly-Sar-Sar (SEQ ID NO:10). However, from a synthetic point of view substitution at the *N*-1 position is less desirable, as this facilitates diketopiperazine formation at the dipeptide stage during assembly of the linear precursor. We have found that altering the position of the backbone substituent can significantly affect the ratio of monocycle over dimer or higher oligomers.

In the specification, from page 31, line 35 to page 32, line 2, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

We have extended this *N*-substitution approach to include reversible *N*-substitution. Three linear precursors, the backbone unprotected peptide X (Asp(OBu)-Val-Gly-leu; SEQ ID NO:14) and two backbone HMB-substituted analogues Y (Asp(OBu)-(HMB)Val-Gly-Leu; SEQ ID NO:15) and Z (Asp(O-Bu)-Val-(HMB)-Gly-Leu; SEQ ID NO:16), were prepared.

In the specification, page 39, lines 1-10, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Synthesis of a difficult cyclic peptide, [cyclo[Ala-Phe-Leu-Pro-Ala]- (SEQ ID NO:17):

H-Ala-Phe-Leu-Pro-Ala-OH (SEQ ID NO:18) was a recently reported example of a sequence which is difficult to cyclise (Schmidt and

Langner, 1997). When subjected to cyclisation conditions, dimers and higher oligmers were generated, but no target cyclopentapeptide was formed. In the following set of experiments, summarized in Scheme 7, we demonstrate that the monocycle was accessible using a ring contraction strategy.

In the specification, at page 39, lines 12-19, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Cyclisation of unsubstituted Ala-Phe-Leu-Pro-Ala.

As a control experiment we attempted to cyclise the unsubstituted linear peptide (Ala-Phe-Leu-Pro-Ala; SEQ ID NO:18) using standard cyclisation conditions (1mM in DMF, 3eq. BOP, 5eq. DIEA, 3h at rt). As expected from the previously reported results (Schmidt and Langer, 1997), only cyclic dimer and some trimer were obtained, but no target monocyclic product was isolated.

In the specification, at page 40, lines 6-10 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Scheme 7: Cyclisation of auxiliary containing peptides **1,2** (A) and formation of the target cyclic peptides **7,8**(B) ; i) 3 eq. BOP / 5 eq. DIEA, 3h at rt; ii) 1 eq. BOP / 2 eq. DIEA, 3h rt; 10 eq. DIEA, 12h rt or 1h at 65°C; iii) hν(366nm). Ala-Phe-Leu-Pro-Ala is SEQ ID NO:18 and Phe-Leu-Pro-Ala-Ala is SEQ ID NO:20.

In the specification, at page 41, lines 24-26, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Similarly *N*-(6-nitro-2-hydroxybenzyl)Phe-Leu-Pro-Ala-Ala **2c** (SEQ ID NO:19) was assembled and cyclised as above. The all-L cyclo pentapeptide 8c was isolated in 45% yield.

In the specification, at page 45, lines 1-2 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Application Of Ring Contraction Auxiliary (Scheme 6)

$NH_2CH_2CH_2SSCH_2CH_2$-Gly-Arg-Pro-Phe-Gly-OH (SEQ ID NO:21)

In the specification, from page 45, lines 10 to page 46, line 6 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The peptide $NH_2CH_2CH_2SSCH_2CH_2$-Gly-Arg-Pro-Phe-Gly-OH (SEQ ID NO:21) was synthesised in stepwise fashion from Boc-Gly-Pam resin (0.5 g, 0.5 mmol/g) by established methods, using *in situ* neutralisation/HBtU activation protocols for Boc chemistry. The Pmc protecting group was used for the Arg residue. Coupling reactions were monitored by quantitative ninhydrin assay and were typically >99.9%. After chain assembly was complete and the N-Boc group removed with neat TFA (2 x 1 min treatment) and neutralised with 10% DIEA in DMF (2 x 1 min treatment), the peptide was bromoacetylated by the method of Robey (Robey, F.A., Fields, R.L., Anal. Biochem., 1989 177 373-377). Bromoacetic acid (277.9 mg, 2.0 mmol) was dissolved in CH_2Cl_2 (2 mL), to which was added DIC (126.2 mg, 1 mmol). After activation for 10-15 min to form the symmetric anhydride, the mixture was diluted with DMF (2 mL), added to the peptide resin, and coupled for 30 min. The resin was washed with DMSO, and cystamine (2 M in DMF, 4 mL) was allowed to react with the bromoacetylated peptide resin for 16 h. The linear peptide was cleaved from resin by the

addition of thiocresol: cresol, 1:1 (1 mL), followed by treatment with HF (10 mL) for 1 h at -5°C. After removal of the HF under reduced pressure, the crude peptide was precipitated in anhydrous Et_2O and filtered to remove the scavengers. The peptide was dissolved in HOAc: H_2O, 1:19, filtered and the filtrate lyophilized. $NH_2CH_2CH_2SSCH_2CH_2$-Gly-Arg-Pro-Phe-Gly-OH was purified by semi-preparative HPLC (20-80% B over 60 min) to give the wanted material (79.6 mg 47%) yield. MS $[M+H]^+$ = 668.1 (expected 668.3).

In the specification, at page 46, line 8, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

$HSCH_2CH_2$-Gly-Arg-Phe-Gly-OH (SEQ ID NO:22)

In the specification, at page 46, lines 15-24, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The disulfide (66.8 mg, 0.10 mmol) was dissolved in a 0.03 M solution of $NH_4^+OAc^-$ (20 mL). Tris(2-carboxyethyl)phosphine hydrochloride salt (TCEP) (35.6 mg, 0.15 mmol) was added portionwise to the stirred solution at r.t. After a further 3h at this temperature the resulting mixture was lyophilized to give a white powder. The peptide $HSCH_2CH_2$-Gly-Arg-Phe-Gly-OH (SEQ ID NO:22) was purified by semi-preparative HPLC (20-80% B over 60 min) to yield a white powder (40.1 mg, 68%); MS $[M+H]^+$ = 593.1 (expected 593.3).

In the specification, at page 47, line 1, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Cyclo-(SCH_2CH_2-Gly-Arg-Pro-Phe-Gly) (SEQ. ID NO:23)

In the specification, at page 47, lines 8-17, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The linear peptide $HSCH_2CH_2$-Gly-Arg-Pro-Phe-Gly-OH (SEQ ID NO:24) (40.0 mg, 0.068 mmol) and BOP (88.4 mg, 0.2 mmol) was stirred in DMF (68 mL, $1x10^{-3}$ M) at -10°C. DIPEA (121 µL, 0.68 mmol) was added dropwise to the solution. The reaction was left to stir for a further 2 h at this temperature, before all volatiles were removed *in vacuo*. The peptide Cyclo-(SCH_2CH_2-Gly-Arg-Pro-Phe-Gly) (SEQ ID NO:23) was purified by semi-preparative HPLC (20-80% B over 60 min) to yield a white powder (12.2 mg, 31%); MS $[M+H]^+$ = 743.2 (expected 743.4092).

In the specification, at page 48, line 1, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Bis-[cyclo-Gly(CH_2CH_2S)-Arg-Pro-Phe-Gly] (SEQ ID NO:23)

In the specification, at page 48, lines 8-15, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The peptide Cyclo-(SCH_2CH_2-Gly-Arg-Pro-Phe-Gly) (SEQ ID NO:23) (12 mg, 0.016 mmol) was dissolved in a solution of

Na_2HPO_4 (0.03 M) and stirred at room temperature overnight. The resulting solution was lyophilized to give a white powder. The peptide Bis-[cyclo-Gly(CH_2CH_2S)-Arg-Pro-Phe-Gly] (SEQ ID NO:23) was purified by reverse phase HPLC (20-80% B over 60 min) to yield a white powder (7.4 mg, 81%); MS $[M+2H]^{2+}$ = 574.22 (expected 574.27).

In the specification, at page 49, line 1, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Cyclo-(Gly(CH_2CH_2SH)-Arg-Pro-Phe-Gly) (SEQ ID NO:25)

In the specification, at page 49, lines 8-16, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The disulfide (7.4 mg, 6.50 µmol) was dissolved in a 0.03 M solution of $NH_4^+OAc^-$ (20 mL). TCEP (4.75 mg, 20.0 µmol) was added portionwise to the stirred solution at r.t. After a further 3h at this temperature the resulting mixture was lyophilized to give a white powder. The peptide Cyclo-(Gly(CH_2CH_2SH)-Arg-Pro-Phe-Gly) (SEQ ID NO:25) was purified by semi-preparative HPLC (20-80% B over 60 min) to yield a white powder (5.5 mg, 74%); MS $[M+H]^+$ = 575.24 (expected 575.28).

In the specification, at page 49, lines 18-19, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Experimental to synthesis of cyclo [Ala Phe Leu Pro Ala] (SEQ ID NO:17) Cyclisation experiments.

In the specification, at page 50 lines 5-25, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Cyclo-[*N*-(5-nitro-2-hydroxybenzyl)-Ala-Phe-Leu-Pro-Ala] (7a) (SEQ ID NO:26). Cyclisation of *N*-(5-nitro-2-hydroxybenzyl)-Ala-Phe-Leu-Pro-Ala **1a** (SEQ ID NO:26) (30 mg of the TFA salt, 0.038 mmol), produced **7a** (12.5 mg, 0.019 mmol) in 51% yield : ES-MS *Mr* 650.2, calcd for $C_{33}H_{42}N_6O_8$, 650.3 (monoisotopic). ^1H NMR (500 MHz, DMSO-d_6, ppm) δ 11.5 (s, 1H, OH), 8.40 (d, 1H, N*H*$_{Leu}$), 8.02 (dxd, 1H, *H*-ar), 7.70 (d, 1H, *H*-ar), 7.4 (d, 1H, *H*N$_{Phe}$), 7.20-7.30 (m, 5H, *H*-Phe), 6.99 (d, 1H, *H*-ar), 6.54 (d, 1H, *H*-N$_{Ala}$), 5.00 (s, 1H, ArCH*h*N-), 4.91 (m, 1H, α-Ala^5), 4.75 (q, 1H, α-Ala^1), 4.59 (m, 1H, α-Phe), 4.50 (m, 1H, α-Leu), 4.27 (t, 1H, α-Pro), 3.88 (d, 1H, ArCH*h*N-), 3.62 (m, 1H, δ-Pro), 3.37 (m, 1H, δ-Pro), 2.97 (m, 1H, β-Phe), 2.82 (m, 1H, β-Phe), 2.04 (m, 2H, β-Pro), 1.88 (m, 1H, γ-Pro), 1.73 (m, 1H, β-Leu), 1.65 (m, 1H, γ-Pro), 1.44 (m, 1H, γ-Leu), 1.33 (m, 1H, γ-Leu), 1.24 (d, 3H, β-Ala^5), 0.91 (d, 3H, β-Ala^1), 0.85 (m, 6H, δ-Leu). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) 172.61, 170.34, 170.07, 169.95, 169.47, 160.40, 139.73, 136.88, 129.31, 128.14, 126.50, 125.72, 124.21, 122.65, 115.00, 61.04, 56.50, 55.74, 48.70, 46.31, 44.34, 41.37, 38.28, 31.30, 24.20, 22.81, 22.68, 21.17, 18.97, 15.35.

In the specification, at page 50, lines 27-36 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Cyclo-[*N*-(6-nitro-2-hydroxybenzyl)-Ala-Phe-Leu-Pro-Ala] (8a) (SEQ ID NO. 27). From cyclisation of *N*-(6-nitro-2-hydroxybenzyl)-Ala-Phe-Leu-Pro-Ala **2a** (SEQ ID NO. 27) (20 mg of the TFA salt, 0.025 mmol), **8a**(6.5 mg, 0.010 mmol) was obtained in 39% yield : ES-MS *Mr* 650.6, calcd for $C_{33}H_{42}N_6O_8$: 650.3 (monoisotopic). ^{13}C NMR (75 MHz, CD_3OD, ppm) δ 178.07, 176.95, 174.54, 174.32, 173.72, 159.11, 153.19, 140.41, 131.99, 129.96, 129.54, 127.57, 121.18, 116.57, 62.75, 60.67, 58.55, 54.05, 51.15, 44.54, 43.41, 34.85, 33.67, 25.03, 24.13, 22.30, 21.31, 15.49, 13.89.

In the specification, at page 51, lines 1-10 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Cyclo-[*N*-(6-nitro-2-hydroxybenzyl)-Phe-Leu-Pro-Ala-Ala] (8c) SEQ ID NO:28). From cyclisation of the *N*-(6-nitro-2-hydroxybenzyl)-Phe-Leu-Pro-Ala-Ala (SEQ ID NO:28 (20 mg of the TFA salt, 0.025 mmol), **8a**(7.3 mg, 0.011 mmol) was obtained in 44% yield : ES-MS *Mr* 650.2, calcd for $C_{33}H_{42}N_6O_8$: 650.3 (monoisotopic). ^{13}C NMR (75 MHz, DMSO-d6, ppm) δ 171.43, 171.00, 169.46, 167.56, 156.65, 138.43, 129.24, 129.05, 128.32, 128.18, 126.08, 119.50, 115.87, 114.60, 62.18, 60.69, 51.07, 49.38, 46.57, 45.46, 41.54,38.17, 33.65, 31.43, 24.37, 22.73, 22.32, 21.06, 17.87, 16.92.

In the specification, at page 51, lines 12-27 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Cyclo-[Ala-Phe-Leu-Pro-Ala] (9a) (SEQ ID NO:17). a) Cyclo-[*N*-(6-nitro-2-hydroxybenzyl)-Ala-Phe-Leu-Pro-Ala] (SEQ ID NO:27 (1mM

MeOH) was purged with nitrogen for 30 minutes and then photolysed with a standard laboratory UV lamp (366nm, 0.25A) for three hours. The MeOH was evaporated and the residue dissolved in 50% buffer B, and the solution loaded directly onto a Vydac C18 column (preparative) for HPLC purification. Cyclo-[Ala-Phe-Leu-Pro-Ala] (SEQ ID NO:17) was isolated in 52% yield. The product coeluted with an independently synthesised sample. ES-MS *Mr* 499.4, calcd for $C_{26}H_{37}N_5O_5$, 499.3 (monoisotopic). b) Photolysis of purified cyclo-[*N*-(6-nitro-2-hydroxybenzyl)-Phe-Leu-Pro-Ala-Ala] (SEQ ID NO:28) was perfomed as described above. Cyclo-[Phe-Leu-Pro-Ala-Ala] (SEQ ID NO:30) was isolated in 28% yield. The product coeluted with a independently synthesised sample. ES-MS *Mr* 499.1, calcd for $C_{26}H_{37}N_5O_5$, 499.3 (monoisotopic).

In the specification, at page 52, lines 16-21 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

1a. **[Hnb]**Tyr-Arg-Phe-Gly (SEQ ID NO:31)

1b. Tyr-**[Hnb]**Arg-Phe-Gly (SEQ ID NO:32)

1c. Tyr-Arg-**[Hnb]**Phe-Gly (SEQ ID NO:33)

1d. **[Hnb]**Tyr-**[Hnb]**Arg-Phe-Gly (SEQ ID NO:34)

1e. **[Hnb]**Tyr-Arg-**[Hnb]**Phe-Gly (SEQ ID NO:35)

In the specification, at page 53, lines 16-30 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

In contrast, for peptides **1d** and **1e**, which contain both a backbone substitutent and a ring contraction auxiliary, ring closure and ring contraction was almost complete under the same mild reaction conditions (6h at rt). Figure 5 shows the

cyclisation profiles of peptides **1a**, **1d** and **1e** after 6h at rt. Under these mild conditions, peptide **1a** did not undergo any significant ring contraction, and the crude product contained largely linear peptide (L). Peptides **1d** and **1e** on the other hand produced the target cyclic peptides cyclo-[(Hnb)Tyr-(Hnb)Arg-Phe-Gly] **2d** (SEQ ID NO:36) and cyclo-[(Hnb)Tyr-Arg-(Hnb)Phe-Gly] **2e** (SEQ ID NO:37) respectively (MW: calcd for $C_{40}H_{43}N_9O_{11}$ = 825.3 (monoisotopic), exp (Cycl peptide **2d)** = 825.1, exp (Cycl peptide **2e)** = 825.1) in excellent purity and yield . Note that the cyclic products have the same molecular weight but different substitution patterns.

In the specification, at page 54, lines 14-16 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

1f. **[Hnb]**Gly-**[Hnb]**Tyr-Arg-Phe (SEQ ID NO:38)

1g. **[Hnb]**Gly-Tyr-**[Hnb]**Arg-Phe (SEQ ID NO:39)

1h. **[Hnb]**Gly-Tyr-Arg-**[Hnb]**Phe (SEQ ID NO:40)

In the specification, at page 55, lines 18-30 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

A large scale cyclisation was performed on peptide **1f**,and two cyclic products were isolated by HPLC as a mixture in 68% yield (by weight). The two products could be separated by HPLC and photolysed to generate one unsubstituted cyclic peptide each (MW = 523 gr/mol) (non-coeluting). One of the products coeluted with the product from peptide **1d**, and therefore was assigned to be the all-*L* cyclo-[Gly-Tyr-Arg-Phe] (SEQ ID NO:41). The second eluting product was assigned to be the cyclo-[Gly-Tyr-Arg-(*D*)phe] (SEQ

ID NO:41). Photolysis of the mixture generated a mixture of the two cyclic unsubstituted peptides in 34% yield (overall yield 23%). The first product coelutes with the product obtained by cyclisation and subsequent photolysis of peptide **1d**.

In the specification, at page 55, lines 32-34 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Combination of ring contraction and backbone substitution for the synthesis of cyclo-[Tyr-Arg-Phe-Ala] (SEQ ID NO:42), with cyclisation at the Tyr-to-Ala site.

In the specification, from page 55, line 36 to page 56, line 7, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

As mentioned in the background section of this specification, turn-inducing elements such as Gly and Pro can favour cyclisation. Here we apply our combination technology to the synthesis of peptides that do not contain turn-inducing amino acids. In this example we employ the combination strategy (backbone substitution and ring contraction auxiliaries) for the synthesis of a very difficult target, an all-L cyclic tetrapeptide cyclo-[Tyr-Arg-Phe-Ala] (SEQ ID NO:42).

In the specification, page 56, line 10, please delete the existing line and replace with the following line after implementing the following changes:

4 [Hnb] Tyr-Arg- **[Hnb]** Phe-Ala (SEQ ID NO:43)

In the specification, page 57, lines 21-36, please delete the existing line and replace with the following line after implementing the following changes:

Product A: Unstable to heat; the product fully decomposed upon heating for 20 h at 70°C in DMSO. Stable to hydrolysis (aqueous buffer at pH 9).
Photolysis of this compound in DMSO proceeded reasonably well; both HnB groups were removed, and **cyclo-[Tyr-Arg-Phe-(*D*)Ala]** (SEQ ID NO:42) was isolated by HPLC in 42% yield.
The presence of *D*-Ala was confirmed by chiral amino acid analysis.

Product B: Stable to heat and to hydrolysis conditions aqueous buffer at pH 9).
Photolysis did not proceed very readily.
Chiral amino acid analysis confirmed the presence of *L*-Ala.
This product is the all-*L* cyclo-[(Hnb)Tyr-Arg-(Hnb)Phe-Ala] (SEQ ID NO:44).

In the specification, at page 58, line 7, please delete the existing line and replace with the following line after implementing the following changes:

5. **[Hnb]**Ala-Tyr-**[Hnb]**Arg-Phe (SEQ ID NO:45)

In the specification, from page 59, line 32 to page 60, line 7, please delete the existing line and replace with the following line after implementing the following changes:

Large scale cyclisation of peptide 1d: 0.011 mmol of linear peptide **1d** (10 mg of the TFA salt) was dissolved in DMF (5mL) containing 0.012 mmol BOP (5.2 mg). DMF (5mL) containing 0.025 mmol DIEA (4.3 μL) was added, and the mixture stirred for 3 hours (rt). 0.25 mmol DIEA (40μL) was added and the reaction left stirring for another 20 hours. The solvent was evaporated under high vacuum, the residue dissolved in acetonitrile/water and loaded on a preparative HPLC column. A 1.5 % gradient was used to elute the products (100% buffer A to 20% buffer A). Cyclo-[(Hnb)Tyr-(Hnb)Arg-Phe-Gly] **2d** (SEQ ID NO:46) (5.3mg, 0.0064 mmol, 61%) was isolated: ES-MS: calcd for $C_{40}H_{43}N_9O_{11}$ = 825.3 (monoisotopic), exp = 825.1.

In the specification, at page 60, lines 9-18 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The product **2d** (5 mg, 6 x 10^{-3} mmol) was then dissolved in DMF (10mL), the solution placed in a beaker and photolysed for 3 hours using a UV lamp (350 - 365nm, 20W, Black/White/Blue). The DMF was removed under vacuum, the residue dissolved in acetonitrile/water, the solution filtered and loaded on a preparative HPLC column. A 1.5% gradient from 100%A to 20%A was used to elute the products. Cyclo-[Tyr-Arg-Phe-Gly] (SEQ ID NO:47) was isolated in 47% yield (1.5 mg, 2.8 10^{-3} mmol) : ES-MS: calcd for $C_{26}H_{33}N_7O_5$ = 523.2 (monoisotopic), exp = 523.3.

In the specification, at page 61, lines 7-30 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Large scale cyclisation of peptide 1f: Peptide **1f** (30 mg of the TFA salt, 0.0355 mmol) was dissolved in DMF (30 mL) and 6 eq DIEA

(18.3 μL) added. After addition of 1 eq BOP (17.1 mg) the reaction was stirred for 20 h. The solvent was then removed (high vacuum), the residue dissolved in acetonitrile/water and the solution loaded directly onto a preparative HPLC column. A 1.5% gradient from 100%A to 20%A was used to elute the products. The fractions containing cyclic product were collected, combined and lyophilised. 17.5 mg of a mixture of two products was obtained (68% yield): ES-MS: Calcd for $C_{40}H_{43}N_9O_{11}$ = 825.3 (monoisotopic), Exp = 825.1. The mixture of two products (17 mg) was dissolved in DMF (20mL) and photolysed for 3 hours. The solvent was removed, the residue dissolved in acetonitrile/water and the solution loaded onto a preparative HPLC column. A 1.5% gradient from 100%A to 20%A was used to elute the products. The target cyclic products, cyclo-[Gly-Tyr-Arg-(*L*)Phe] (SEQ ID NO:48) and cyclo-[Gly-Tyr-Arg-(*D*)Phe] (SEQ ID NO:48) were isolated as a mixture (3.8 mg, 35% yield): ES-MS: calcd for $C_{26}H_{33}N_7O_5$ = 523.2 (monoisotopic), Exp = 523.3. The ratio of *L*-Phe/*D*-Phe was determined by chiral amino acid analysis to be 2/3. Of the mixture of two cyclic products, the first eluting one coeluted with the all-*L* cyclo-[Tyr-Arg-Phe-Gly] 1d (SEQ ID NO:47) synthesised as described above.

In the specification, at page 61, lines 32-34 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Combination of ring contraction and backbone substitution for the synthesis of cyclo-[Tyr-Arg-Phe-Ala] (SEQ ID NO:42), cyclisation at the Tyr-to-Ala site.

In the specification, at page 62 lines 17-27 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Product A (9mg, 18 %) ES-MS: calcd for $C_{41}H_{45}N_9O_{11}$ = 839.3 (monoisotopic), exp = 839.5. Chiral amino acid analysis of the product showed the presence of *L*-Tyr, *L*-Arg, *L*-Phe and *D*-Ala. Product A = cyclo-[(Hnb)Tyr-Arg-(Hnb)Phe-(*D*)Ala] SEQ ID NO:44). **Product B** (7mg, 13%) ES-MS: calcd for $C_{41}H_{45}N_9O_{11}$ = 839.3 (monoisotopic), exp = 839.5. Chiral amino acid analysis showed the presence of *L*-Tyr, *L*-Arg, *L*-Phe and *L*-Ala. Product B = cyclo-[(Hnb)Tyr-Arg-(Hnb)Phe-Ala] (SEQ ID NO:44). Another 8 mg of a mixture of products A and B (15%) was isolated , giving a total cyclisation yield of 46%.

In the specification, from page 62, line 29 to page 63, line 2 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Photolysis of cyclo-[(Hnb)Tyr-Arg-(Hnb)Phe-(*D*)Ala] (SEQ ID NO:44): Product A (9 mg) was dissolved in DMF (100 mL) and photolysis carried out for 3h. The solvent was removed, the residue dissolved in acetonitrile/water and the solution loaded onto a preparative HPLC column. A 1.5% gradient from 95%A to 10%A was used to elute the products. The cyclic product, cyclo-[Tyr-Arg-Phe-(*D*)Ala] (SEQ ID NO:42) was isolated (2.4 mg, 42% yield): ES-MS: calcd for $C_{27}H_{35}N_7O_5$ = 537.61 (monoisotopic), exp = 537.2. Chiral amino acid analysis of this product showed presence of *L*-Tyr, *L*-Arg, *L*-Phe and *D*-ALa.

In the specification, at page 63, lines 14-16 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Cyclo-[(Hnb)Tyr-Arg-(Hnb)Phe-Ala] (SEQ ID NO:44): (15.6mg, 60%) ES-MS: calcd for $C_{41}H_{45}N_9O_{11}$ = 839.3 (monoisotopic), exp = 839.2.

In the specification, at page 65, lines 16-19, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

[Linear] tetrapeptide	Yield of Cyclisation
cyclo-[DG-Act-RG] (SEQ ID NO:1)	11%
cyclo-[DG-Amb-RG] (SEQ ID NO:50)	7%; 3% dimer
cyclo-[D-Amb-GRG] (SEQ ID NO:51	5% monomer; 5% dimer

In the specification, at page 65, lines 22-24, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

This section describes the experimental details for the synthesis of the activated linker and model peptides. Linear DG-Act-RG is SEQ ID NO:49.

In the specification, at page 65, lines 25-26 please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Synthesis of Model Compounds Using Activated Linkers Cyclo [DGActRG] (SEQ ID NO. 1) (Table 5)

In the specification, from page 66, line 33 to page 67, line 2, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The resin was stirred in DMF (10 mL) containing DIEA (100 µL) for 12 hours. The resin was filtered off and the DMF removed *in vacuo*. The residue was dissolved in a minimal amount acetonitrile/water (1/1) and loaded directly on a preparative reverse phase column for HPLC separation of the product. Cyclo-[DGCatRG] (SEQ ID NO:1) (27 mg, 11% yield from the starting resin) was obtained.

In the specification, at page 67, lines 3-6, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The same protocols were followed to assemble, deprotect and cyclise the following peptides: cyclo-[DGAmbRG] (SEQ ID NO:50): 7.6% yield (3% dimer); cyclo-[DAmbGRG] (SEQ ID NO:51): 5% yield (5% dimer).

In the specification, at page 83, line 16, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Solid-Phase Synthesis of cyclo-[D-G-Amb-R-G] (SEQ ID NO:50)

In the specification, from page 83, line 22 to page 84, line 3, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The benzyloxy linker (429 mg, 1.0 mmol, 2.0 equiv.) was coupled using standard HBTU/DIPEA protocols overnight. The remaining residues were coupled using standard HBTU/DIPEA protocols for ten minutes each. The final yield of the dried resin was 906 mg. Of this, 725 mg (ca. 0.4 mmol) was cleaved with anhydrous HF using anisole as the scavenger. The resin was

washed well with diethyl ether, dried at suction, then gently stirred in 5.0 cm^3 DMF containing 0.5 cm^3 DIPEA for 48 h. The resin was filtered off and washed well with DMF. Evaporation of the filtrate, followed by preparative HPLC gave *cyclo*-[D-G-Amb-R-G] (SEQ ID NO:50) as a fluffy white solid (103 mg, 49%). Analysis of the product by LC-MS indicated the presence of the cyclodimer, *cyclo*-[D-G-Amb-R-G-D-G-Amb-R-G] (SEQ ID NO:52). The ratio of monomer to dimer was approximately 3:2.

In the specification, at page 84, lines 10-21, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

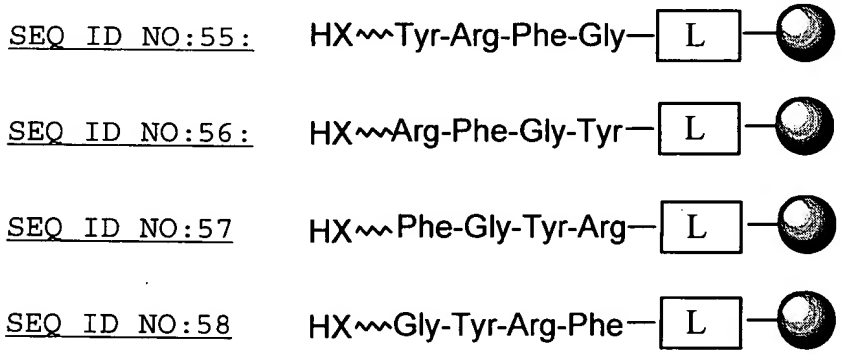
The sequence Ala-Phe-Leu-Pro-Ala (SEQ ID NO:18) does not cyclize under solution conditions (Schmidt and Lagner, 1997) using BOP/DIEA or under on-resin conditions using the safety-catch linker. However, when the backbone substitution method is applied in combination with the safety-catch linker a substantial amount of cyclic product is obtained. For example, the synthesis and cyclisation of Ala-(Me)Phe-Leu-Pro-Ala (SEQ ID NO:53) yields cyclic product as characterised by ES-MS. Although in this instance the backbone substitution was a methyl group, one skilled in the art would realise that numerous other substituents may also be used, including reversible substituents such as HMB and HnB.

In the specification, from page 84, line 24 to page 85, line 4, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The assembly of the peptide was carried out using standard *in situ* neutralization Boc-SPPS protocols on aminomethylated polystyrene resin (sv=0.26meq/g) derivatised with the safety-catch linker as previously described (see Example 5). After

coupling of Boc-(Me)Phe-OH and removal of the Boc group, the peptide was acylated using a solution of the symmetric anhydride of Boc-Ala, prepared from Boc-Ala (10eq) and DIC (5eq) in DCM. The resin was then treated with TFMSA/TFA/p-cresol (1:10:1) for 2h to remove the benzyl group for linker activation. The resin was then washed with TFA (3 x 10mL), DCM (3 x10mL) and DMF (3 x10mL). The resin was then treated with 2% DIEA in DMF overnight. The solvent was removed on the Genevac and the residue resuspended in acetonitrile/water and analyzed by ES-MS and reversed phase HPLC. The ES-MS spectrum displayed a major peak at the expected m/z value for the cyclo-[Ala-(Me)Phe-Leu-Pro-Ala] (SEQ ID NO:54) calculated for $C_{27}H_{39}N_5O_5$ = 513.3 (monoisotopic), exp = 513.3.

In the specification, at page 85, lines 17-18, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:



In the specification, at page 86, lines 5-11, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

In this example we show that the combination of ring contraction and backbone substitution can also be applied in an on-resin cyclisation strategy. The selected sequence, [Hnb]Gly-[Hnb]Tyr-Arg-Phe (SEQ ID NO:38), cyclises readily in solution, as illustrated in Example 3. We have applied our safety-catch linker (Example 5) to generate the target cyclic peptide directly from resin.

In the specification, from page 86, line 15 to page 87, line 19, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The assembly of the peptide was carried out on Boc-Phe-Linker-resin, which was synthesised in the standard manner (see example 6; the resin was aminomethylated resin, sv=0.26meq/gr) . The peptide was then assembled using *in situ* neutralisation protocols and Boc-SPPS as described previously . The Hnb group was introduced using the standard reductive amination approach. Special care was taken to minimise the time of exposure to $NaBH_4$ (1 eq of $NaBH_4$ for 1 min), as this can cause premature cleavage of the peptide from the resin. After introduction of the first Hnb group, Boc-Gly was attached via its HBTU activated ester (overnight). The resin was further treated with 1% piperidine (5 min) to remove the *O*-acylation on the phenol (Hnb). Following introduction of the second HnB group as described above, the resin was treated with HF/p-cresol (9/1; 1h at 0°C) to remove the side-chain protection groups and the benzyl group for linker activation. The resin was then washed with ether (3 x 10 mL), DMF (3 x 10 mL), DCM/MeOH (10 mL) and dried under high vacuum for 2h. The resin was then treated with 1% DIEA in DMF overnight. After removal of the solvent, the residue was resuspended in acetonitrile/water and analysed by ES-MS and reversed phase HPLC. The ES-MS spectrum displayed a major peak at the expected m/z value for the cyclo-[[Hnb]Gly-[Hnb]Tyr-Arg-Phe] (SEQ ID NO:2)

(calculated for $C_{40}H_{43}N_9O_{11}$ = 825.3 (monoisotopic), exp M = 825.4 gr/mol).

In the specification, from page 90, line 12 to page 91, line 4, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The linear precursor sequences were then assembled on resin using *in situ* neutralisation protocols. Removal of the *C*-terminal allyl protection group was accomplished using $Pd(Ph3P)_4$. The resin-bound linear peptide was further cyclised using BOP/DIEA activation. After deprotection and cleavage (HF), products were separated, analysed and weighed. The reaction products consisted mainly of cyclic monomer and cyclic dimer. The results are shown in Table 6, in which the amino acid sequence is given in single-letter code. PFNSLAI is SEQ ID NO:59 and NSLAIPF is SEQ ID NO:60.

In the specification, at page 100, line 1, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

H-Asn-Ser-Leu-Ala-Ile-Pro-Phe-OH (SEQ ID NO:60)

In the specification, at page 101, line 1, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

H-Pro-Phe-Asn-Ser-Leu-Ala-Ile-OH (SEQ ID NO:59)

In the specification, at page 101, lines 8-14, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The peptide was synthesised using a similar procedure to that in the previous experiment above using the precursor Boc-Ala-[Backbone attachmenet]-Ile-O-Allyl (200 mg, 0.180 mmol/g). The peptide H-Pro-Phe-Asn-Ser-Leu-Ala-Ile (SEQ ID NO:59) was purified by semi-preparative HPLC (30-90% B over 60 min) to yield a white powder (10.5 mg, 39%); MS $[M+H]^+$ = 761.2 (expected 761.4).

In the specification, at page 101, line 17, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Method 1: Cyclo-(Pro-Phe-Asn-Ser-Leu-Ala-Ile) (SEQ ID NO:63)

In the specification, from page 101, line 24 to page 102, line 13, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The linear peptide H-Asn-Ser-Leu-Ala-Ile-Pro-Phe-OH (SEQ ID NO:60) (15.0 mg, 0.020 mmol) and BOP (26.1 mg, 0.060 mmol) was stirred in DMF (19.7 mL, $1x10^{-3}$ M) at -10°C. DIPEA (35 µL, 0.197 mmol) was added dropwise to the solution. After the reaction was left to stir for a further 2 h at this temperature, all volatiles were removed *in vacuo*. The peptide Cyclo-(Pro-Phe-Asn-Ser-Leu-Ala-Ile) (SEQ ID NO:63) was purified by semi-preparative HPLC (30-90% B over 60 min) to yield a white powder (7.0 mg, 48%). ^{1}HNMR (DMSO): δ MS $[M+H]^+$ = 743.2 (expected 743.4092). Also isolated was the dimer, Cyclo-(Asn-Ser-Leu-Ala-Ile-Pro-Phe-Asn-Ser-Leu-Ala-Ile-Pro-Phe) (SEQ ID NO:64) (3 mg,

21%); MS $[M+H]^+$ = 1486.2 (expected 1486.8), and the trimer, Cyclo-(Asn-Ser-Leu-Ala-Ile-Pro-Phe-Asn-Ser-Leu-Ala-Ile-Pro-Phe-Asn-Ser-Leu-Ala-Ile-Pro-Phe) (SEQ ID NO:65) (0.7 mg, 5%); MS $[M+H]^{2+}$ = 1115.1 (expected 1115.1)

In the specification, at page 102, line 15, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Method 2: Cyclo-(Pro-Phe-Asn-Ser-Leu-Ala-Ile) (SEQ ID NO:63)

In the specification, from page 102, line 22 to page 103, line 2, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The peptide was synthesized using a similar procedure to Method 1 above using H-Pro-Phe-Asn-Ser-Leu-Ala-Ile-OH (SEQ ID NO:59) (100 mg, 0.131 mmol), BOP (174 mg, 0.393 mmol), and DIPEA (228 µL, 1.31 mmol). The peptide cyclo-(Pro-Phe-Asn-Ser-Leu-Ala-Ile) (SEQ ID NO:63) was purified by semi-preparative HPLC (10-70% B over 60 min) to yield a white powder (10.5 mg, 67%); MS $[M+H]^+$ = 743.2 (expected 743.4092). All other physical characteristics (^1H NMR, m.p., HPLC retention time, and amino acid analysis) were also consistent with the results reported for Method 1.

In the specification, at page 103, line 5, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Method 1: Cyclo-(Pro-Phe-Asn-Ser-Leu-Ala-Ile) (SEQ ID NO:63)

In the specification, from page 103, line 12 to page 104, line 9, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

After chain assembly for the linear peptide was complete (synthesised from the solid support where the linker was attached between Boc-Pro-Phe-O-Allyl). The allyl protecting group and the N^{α}-Boc group was removed with $[Pd(PPh_3)_4]$ (580 mg, 0.5 mmmol) and TFA (2 x 1 min treatment) the reaction mixture was then cooled to -10°C and BOP (221 mg, 0.5 mmol) was added. 2,6 Lutidene (194 µL, 1.66 mmol) was then added dropwise and the reaction continued until the ninhydrin assay found an absence of amine <0.1%. The organic material was filtered from the resin (250 mg, 0.167 mmol/g) and the cyclic peptide was cleaved from resin using HF : p-cresol, 11 mL, 10:1, for 1 h at -5°C. After removal of the HF under reduced pressurre, the crude peptide was precipitated in anhydrous ether before being dissolved in the HPLC buffer and lyophilized. The peptide Cyclo-(Pro-Phe-Asn-Ser-Leu-Ala-Ile) (SEQ ID NO:63) was purified by semi-preparative HPLC (30-90% B over 60 min) to yield a white powder (3.1 mg, 10%): ^{1}HNMR (DMSO) δ MS $[M+H]^+$ = 743.2 (expected 743.4092). Also isolated was the dimer, Cyclo-(Asn-Ser-Leu-Ala-Ile-Pro-Phe-Asn-Ser-Leu-Ala-Ile-Pro-Phe) (SEQ ID NO:64) (7.6 mg, 24.5%); MS $[M+H]^+$ = 1486.2 (expected 1486.8), and the trimer, Cyclo-(Asn-Ser-Leu-Ala-Ile-Pro-Phe-Asn-Ser-Leu-Ala-Ile-Pro-Phe-Asn-Ser-Leu-Ala-Ile-Pro-Phe) (SEQ ID NO:65) (0.4 mg, 1%); MS $[M+H]^{2+}$ = 1115.2 (expected 1115.1). All other physical characteristics (^{1}H NMR, m.p., HPLC retention time, and amino acid analysis) were also consistent with what was reported above.

In the specification, at page 104, line 11, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Method 2: Cyclo-(Pro-Phe-Asn-Ser-Leu-Ala-Ile) (SEQ ID NO:63)

In the specification, at page 104, lines 18-28, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The peptide was synthesized using a similar procedure to Method 1 using the precursor where the linker was attached between Boc-Ala-Ile-O-Allyl (200 mg, 0.203 mmol/g), $[Pd(PPh_3)_4]$ (290 mg, 0.250 mmmol), BOP (60 mg, 0.136 mmol), and 2,6-lutidene (237 µL, 2.03 mmol) The peptide cyclo-(Pro-Phe-Asn-Ser-Leu-Ala-Ile) (SEQ ID NO:63) (3) was purified by semi-preparative HPLC (30-90% B over 60 min) to yield a white powder (8.2 mg, 25%); MS $[M+H]^+$ = 743.2 (expected 743.4). All other physical characteristics (^1H NMR, m.p., HPLC retention time, and amino acid analysis) were also consistent with what was reported above.

In the specification, at page 106, please delete Table 7 and replace with the following Table 7 after implementing the following changes:

Table 7

Cyclisation Yields Using Fmoc Backbone Linker

Peptide Sequence	Yield (%)	Reaction Time	SEQ ID NO:
Cyclo-[Leu-Asp-Val-Gly-ß-Ala]	18%	12 h	66
Cyclo-[Arg-Gly-Asp-Gly-ß-Ala]	9%	24 h	67
Cyclo-[Phe-Lys-Trp-Gly-ß-Ala]	15%	12 h	68

In the specification, at page 114, line 1, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Cyclo-[Leu-Asp-Val-Gly-β-Ala] (SEQ ID NO:66)

In the specification, at page 114, lines 8-11, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Cyclo-[Leu-Asp-Val-Gly-β-Ala] (SEQ ID NO:66) was lyophilised to a white powder (12.3 mg, 18%): MS $[M+H]^+$ = 456.3 (456.3); Amino Acid Analysis: Gly = 1.06, β-Ala = 1.01, Asp = 1.03, Val = 1.03, Leu = 0.88.

In the specification, at page 114, line 13, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Cyclo-[Phe-Trp-Lys-Gly-β-Ala] (SEQ ID NO:62)

In the specification, at page 114, lines 20-23, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Cyclo-[Phe-Trp-Lys-Gly-β-Ala] (SEQ ID NO:62) was lyophilised to a white powder (8.1 mg, 9%): MS $[M+H]^+$ = 590.1 (expected 590.3). Amino Acid Analysis: Gly = 0.99, β-Ala = 1.01, Lys = 1.04, Phe = 1.02, Trp = 0.95.

In the specification, at page 115, line 1, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Cyclo-[Arg-Gly-Asp-Gly-β-Ala] (SEQ ID NO:67)

In the specification, at page 115, lines 8-11, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Cyclo-[Arg-Gly-Asp-Gly-β-Ala] (SEQ ID NO:67) was lyophilised to a white powder (8.2 mg, 15%): MS $[M+H]^+$ = 457.1 (457.3). Amino Acid Analysis: Gly = 1.95, β-Ala = 1.01, Asp = 0.96, Arg = 1.09.

In the specification, at page 115, lines 14-15, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Application to the synthesis of cyclo - [Ala Pro Leu Phe Ala] (SEQ ID NO:72)

In the specification, at page 115, lines 16-24, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

As is emphasised below, we have evaluated the combination of the backbone linker and ring contraction approach in the synthesis of cyclo [Ala Pro Leu Phe Ala] (SEQ ID NO:72). In this instance the peptide was assembled on the backbone

linker, and the ring contraction auxiliary appended to the N-terminus through reductive amination. Initial cyclisation and ring contraction were allowed to proceed on resin. The resulting cyclic product was then cleaved off the resin using anhydrous HF.

In the specification, at page 117, lines 1-7, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Scheme 14 Reagents and Conditions: I, H-Gly-Leu-Leu-  HBTU, DIEA, DMF, r.t.; ii, Ala-OAllyl, $NaBH_3CN$, 5% HOAc/MeOH, r.t., 3 h; iv, $(Boc\text{-}Pro)_2\text{-}O$, DCM, r.t., 16 h; iv, SPPS; v, 2-Hydroxy-4-nitro-benzaldehyde, $NaBH_4$, DMF, 2 h; vi, $Pd(Ph_3)_4$, CH_3Cl: HOAc : NMM, 37:2:1, r.t, 3 h; vii DIC, DIEA, 70°C, 2 h;.viii, HF : p-cresol, 10:1, -5 °C, 1 h. Compound 5 is SEQ ID NO:69.

In the specification, at page 117, lines 9-10, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Application to the synthesis of a cyclic tetrapeptide, cyclo[[Hnb]Tyr Arg Phe Gly] (SEQ ID NO:5)

In the specification, at page 118, lines 5-9, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

[a]**Reagents:** i TFA : DCM (40:60), 2 x 5 min; ii, Fmoc-Tyr(Boc)-OH, HBTU, DIEA, DMF, 1 h.; iii, piperidine : DMF, 1:1, 2 x 5 min; iv, HnB 2, $NaBH_4$, DMF, rt, 1 h; v, 3 equiv. $Pd(Ph_3)_4$,

CH_3Cl : HOAc : NMM, 37:2:1, r.t, 3 h; vi HF : p-cresol, 1: 1. Linear Tyr-Arg-Phe-Gly is SEQ ID NO:70.

In the specification, at page 118, lines 12-19, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Addition of Fmoc-Tyr(Boc)-OH to **13** using *in situ* neutralisation protocols and HBTU activation resulted in the linear peptide **14** (Scheme 16). Allyl deprotection of **14** using $Pd(PPh_3)_4$ followed by a final TFA treatment gave the desired linear peptide 15 on resin, while removal of the Fmoc protecting group and reductive amination using HnB and $NaBH_4$ followed once again by allyl removal gave the desired linear peptide 16. [HnB]Tyr-Arg-Phe-Gly is SEQ ID NO:4.

In the specification, at page 119, lines 16-17, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

[a]**Reagents:** i, BOP, DIEA, DMF, r.t. 3h; ii, HF : p-cresol, 1: 1. Cyclo-[Tyr-Arg-Phe-Gly] is SEQ ID NO: 42. Cyclo[[HnB]Tyr-Arg-Phe-Gly] is SEQ ID NO:5.

In the specification, at page 120, line 2, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Synthesis of cyclo [Ala Pro Leu Phe Ala] (SEQ ID NO:72)

In the specification, at page 121, lines 20-21, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

H-Ala-Phe-Leu-Pro-[N-(4-(5-oxyvaleric acid)benzyl)]-L-Alanine allyl ester appended to resin ***5*** (SEQ ID NO:73)

In the specification, at page 122, lines 5-7, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

N-(2-hydroxy-4-nitrobenzyl)-Ala-Phe-Leu-Pro-[N-(4-(5-oxyvaleric acid)benzyl)]-L-Alanine allyl ester appended to resin ***6*** (SEQ ID NO:74)

In the specification, at page 123, line 9, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Cyclo-[N-(2-hydroxy-4-nitrobenzyl)-Ala-Phe-Leu-Pro-Ala] ***10*** (SEQ ID NO:75)

In the specification, at page 123, lines 29-30, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Experimental to the synthesis of a cyclic tetrapeptide cyclo[[Hnb]Tyr Arg Phe Gly] (SEQ ID NO:5)

In the specification, at page 124, lines 30-32, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

H-Tyr-Arg-Phe-Gly-OH 17 (SEQ ID NO:70). The linear peptide was isolated in % yield: ES-MS *Mr* 542.2, calcd for $C_{26}H_{36}N_7O_6$, 542.3 (monoisotopic).

In the specification, at page 124, lines 34-36, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

H-[HnB]Tyr-Arg-Phe-Gly-OH 18 (SEQ ID NO:4). The linear peptide was isolated in % yield: ES-MS *Mr* 693.1, calcd for $C_{33}H_{41}N_8O_9$, 693.3 (monoisotopic).

In the specification, at page 125, lines 1-5, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Cyclo-[[HnB]Tyr-Arg-Phe-Gly] 22 (SEQ ID NO:5). Cyclisation of H-[HnB]Tyr-Arg-Phe-Gly-OH on backbone linker 18 produced the cyclo-[[HnB]Tyr-Arg-Phe-Gly] in % yield. ES-MS *Mr* 675.3, calcd for $C_{33}H_{34}N_7O_8$, 675.3 (monoisotopic).

In the specification, at page 125, lines 15-21, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The peptide outlined below (SEQ ID NO:76) is synthesized using this combined approach. This peptide contains 2 N α-substituents (one is the linker L) and a ring contraction

auxiliary. The peptide is cyclised and the purity and yields of products are examined. Reversible Nα-substitution in replacement of methylation is also investigated.

In the specification, at page 125, lines 26-27, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Example 13 Biological activity of cyclo [Tyr-Arg-Phe-Gly] (SEQ ID NO:42) and cyclo [Tyr-Arg-D-Phe-Gly] (SEQ ID NO:42)

In the specification, at page 126, lines 5-21, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Demorphin is a opioid heptapeptide isolated from the skin of South American frogs, and has the following sequence; {H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH2; SEQ ID NO:71). The tetrapeptide analogues (H-Tyr-D-Ala-Phe-Gly-NH-Y; SEQ ID NO:29) are potent analgesics when administered by intracerebroventricular injection. In Example 3 we synthesised the cyclic tetrapeptides cyclo [Tyr-Arg-Phe-Gly] (SEQ ID NO:42) and cyclo [Tyr-Arg-D-Phe-Gly] (SEQ ID NO:42) designated WP 152 using our combination strategies. Figures 10 and 11 shows the effect of these compounds on the focal extracellular recording of evoked excitary for junction currents (EJC) from visualised sympathetic varicosities, measured as described by (Lavidis (1995)). These results illustrates that the mixture of compounds greatly reduces transmitter release. The effect is reversed by the addition of naloxone, strongly suggesting that one or both of the compounds are potent μ-opiate agonists.

At the end of the specification, please insert the enclosed sequence listing.